Comments of the ACC Hydrocarbon Solvents Panel
Attachment II

Justification for the inclusion of complex C9 aromatic fraction in the draft IRIS assessment for trimethylbenzene

Chemical-Specific Charge Questions

B. Literature Search Strategy/Study Selection
1. **Please comment on the whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of 1,2,3-TMB, 1,2,4-TMB, and 1,3,5-TMB.**

ACC Comments:

In setting out the literature search strategy and criteria for the selection and/or exclusion of studies for the toxicological review of trimethylbenzene, the EPA indicated that certain references were excluded (via manual review) because they either involved the use of complex solvent mixtures or were not available in English. **This is inconsistent, as the Battig et al. (1956) papers, cited as evidence for neurotoxic effects in humans, involved exposure to a mixed solvent (80% mixed trimethylbenzene isomers) and were not published in English**. In essence, employing these criteria eliminated a critical set of data on mixed C9 aromatic fractions, primarily consisting of trimethylbenzene and ethyltoluene isomers (Table 1), that would have enriched the existing database for trimethylbenzene, provided information addressing database insufficiency issues raised by the EPA[1] and also providing for a more robust weight of evidence in the consideration of critical endpoints. **For example, the EPA cites the "lack of a multi-generation reproductive/developmental toxicity study" as a weakness of the database. However, as shown in Table 1, a well-conducted 3-generation reproductive toxicity assay in mice, two developmental toxicity assays (mice and rats), and one developmental neurotoxicity assay (rats) are available for which the test substance was a complex C9 aromatic substance consisting predominantly of isomeric mixtures of trimethylbenzene and ethyltoluenes**. Most importantly, the results from the reproductive/developmental toxicity studies of the complex C9 aromatic substances are virtually identical to those that are available for constituents tested individually (1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, ethyltoluene isomers, propylbenzene isomers, butylbenzene, o-, m- and p-xylene) and are discussed in subsequent sections.

[1] Section 2.1.3, Page 2-13, lines 4 – 5 and 10-11 of the Draft Assessment

Table 1: Available database of studies utilizing complex C9 aromatic substances

Test	Assay or Doses	Results	Reference
Genetic Toxicity	Ames *Salmonella* assay CHO HGPRT forward mutation CHO chromosome aberration CHO -SCE Rat chromosome aberration	All studies negative results for gene mutation (*Salmonella*, CHO/HGPRT mutation) or cytogenetic effects. C9 aromatics unlikely to be genotoxic carcinogen	Schreiner et al., 1989‡
Subchronic Neurotoxicity - Rats	100, 500, 1500ppm (500, 2500, 7500mg/m^3) 6hr/day, 5 days/wk for 90 days	No adverse effects for motor activity, functional observation battery or neuropathology	Douglas et al., 1993‡
3-generation reproductive/Developmental toxicity – Mice Female	100, 500, 1500ppm (500, 2500, 7500mg/m^3) 6hr/day from gestational days 6-15	• 1500ppm – 50% mortality • 500ppm - maternal and fetal body weights reduced • 100ppm – no effects	McKee et al., 1990‡
Developmental toxicity – CFY Rats	600, 1000, 2000 mg/m^3 24 hrs/day from gestational days 7-15	• 2000 mg/m^3 – enlarged liver weight in dams. Increased incidence of internal organ retardations and skeletal retardations in foetus • 1000 mg/m^3 - Increased incidence of internal organ retardations and skeletal retardations in foetus • 600 mg/m^3 – no effects **Note –** The internal organ and skeletal retardations did not appear to be functionally relevant as offspring from the 2000 mg/m^3 dose groups showed no adverse effects when sacrificed at post natal day 90.	Ungvary et al., 1983#
Developmental neurotoxicity – CFY Rats	600, 1000, 2000 mg/m^3 24 hrs/day from gestational days 7-15	Tested – body reflexes (time to correction of gait etc.) at day 21 Open field spontaneous locomotive activity at day 23, 36 and 90 Amphetamine sensitivity at day 37 Association and learning ability assessments – at day 42 Results – the authors found no evidence for developmental neurotoxicity with exposure to C9 aromatic fraction.	Lehotzky et al., 1985#
Reproductive Toxicity Rats 30M,30F/group parental	100, 500, 1500ppm (500, 2500, 7500mg/m^3) 6hr/day, 7 days/wk 10 wks pre-mating, 2 wks	No adverse effects on reproductive parameters. Maternal and offspring	McKee et al., 1990‡

	mating (both sexes) females GD0 to GD20 Females not exposed to postnatal day 4 to weaning at LD21. Offspring began exposure after weaning.	body weight effects at 1500ppm	
Repeated dose toxicity Rats	1800, 3700 or 7400 mg/m^3 5d/week for 13 weeks.	Primary effects were liver and kidney weight increases in female rats at mid and high doses with no adverse pathological correlates. Low grade anemia was observed in all exposed females.	Reported in Clark et al., 1989‡
Repeated dose toxicity Rats[a]	450, 900, 1800 mg/m^3 5d/week for 12 months	Primary effect liver weight increase with no adverse pathologic correlate at 1800 mg/m^3	Clark et al., 1989‡

a- EPA considered this study sufficient to fulfill the repeat dose requirement and did not require an additional repeated dose study in the C9 test rule program.
‡ - Studies were conducted under a 1985 EPA test rule program.
# - Independently conducted studies (in Hungarian).

Although the criteria for study exclusion is stated in Figure LS-1[2], no clear rationale for excluding studies utilizing complex C9 mixtures and/or studies available in languages other than English was provided in the document. However the EPA attempted to address public comments to this regard in Appendices E and F[3]. We respond to the EPA justification with the following points:

- **The compositions of the complex C9 aromatic fractions are known and detailed gas chromatographic analysis of the constituents are available in the study reports. In addition, these constituents are all alkylbenzenes with structural similarity to trimethylbenzene.**
- **The toxicological profile (acute, subchronic and reproductive/developmental) of the complex C9 aromatic fractions is identical to those of the individual constituents.**
- **The manufacture, use and exposure to trimethylbenzene occurs primarily in the form of complex C9 aromatics (as acknowledged by the EPA) and hence the use of the data on complex C9 aromatic fractions is useful in the development of a hazard assessment for trimethylbenzenes.**

[1] Composition and the problem of impurities

One criticism of the studies involving complex C9 aromatics is that they are mixtures of multiple constituents, many of which are unknown. For example, the EPA, in its response to earlier public comments indicated that the complex fraction reported in Douglas et al. (1993), McKee et al.

[2] Literature Search Strategy/Study Selection and Evaluation; Page xlviii of the Draft Assessment
[3] Appendix E and F – Summary of available C9 aromatic hydrocarbon fraction toxicity studies and resolution of public comments; Page E1 – F3 of the Supplement to the Draft Assessment

(1990) and Schreiner et al. (1989) contained up to 6% of unknown C10 constituents while that reported in Clark et al. (1989) was comprised of 9% unidentified impurities[4]. In fact, the EPA had indicated that although a comparison of sufficient toxicokinetic and toxicological similarity had been used to support the adoption of reference values for the individual isomers of trimethylbenzene, such a comparison could not be extended to the C9 aromatic fractions because some of the constituents (such as the C10 constituents) were not identified in the compositional analysis, in reference to the Douglas et al. (1983) study[5]. In addition, the EPA indicated that the Lehotzky et al. (1985) and Ungvary et al. (1983) studies were not included in the toxicological review because the compositional make-up of the test substance was not available.

However, although the detailed compositional analysis for the C9 aromatic fraction was not provided in the published studies, this data was available in the original study reports which were provided to the EPA. Table 2 provides an overview of the composition of three different complex C9 fractions that were used in the studies mentioned in Table 1. In the Douglas et al. (1983) study, the C10 constituents (comprising 8.3% of total mixture) were mainly comprised of isomers of dimethyl-ethylbenzene, isomers of methyl-propylbenzene, isomers of butylbenzene (including 0.82% n-butylbenzene), 1,2,4,5- and 1,2,3,5-tetramethylbenzene, 2% diethylbenzene isomers and 0.02% naphthalene. In other words, the C10 component of the C9 fraction in the Douglas et al. (1983) study was not different from that reported for the Clark et al. (1989) study as shown in Table E-2[6].

From Table 2, the C11 constituent of the complex substance in the Clark et al. (1989) study are most likely < 1% similar to that obtained in the Douglas et al. (1993) study. The 8% unaccounted for in the Clark et al. (1989) study is likely to include isopropylbenzene and other C10 constituents similar to those reported in the Douglas et al. (1993) study but for which proportions were not readily available. Although C10 and C11 constituents were not reported in the Lehotzky and Ungvary studies, the proportions of these constituents could not be > 2% since at least 98% of constituents are accounted for.

The C9 fraction employed in Ungvary et al. (1983) and Lehotzky et al. (1985) did not contain a significant amount of constituents ≥ C10 compared to those in the other studies based on differences in manufacturing processes. While the C9 fractions in the Clark et al. (1989) and Douglas et al. (1993) studies are derived primarily from catalytic reforming of petroleum feedstocks, the fraction used in the Ungvary et al. (1983) and Lehotzky et al. (1985) studies undergo additional refining steps in order to meet product specifications for use as solvents (Firth, 2008). **However, as will be shown in the subsequent section, the presence or absence of the ≥ C10 fraction had no effect on toxicity endpoints where a direct comparison can be**

[4] Appendix E, pages E-1 and E-2 of the Supplement to the Draft Assessment.
[5] Table E-1, page E-1 of the Supplement to the Draft Assessment.
[6] Table E-2, page E-2 of the Supplement to the Draft Assessment.

made between the C9 aromatic fractions and individual trimethylbenzene and ethyltoluene isomers.

Independent of source, the C9 fractions had a similar composition (> 76% trimethylbenzenes and ethyltoluenes with xylene and propylbenzene isomers being the other constituents present at more than trace levels). In addition, the constituents present in minute proportions appear to be well characterized and are also structurally similar to trimethylbenzenes (alkylated benzenes), such that a comparison of toxicokinetic and toxicological similarity can be made.

Table 2: Composition of complex C9 fractions used in various mixed C9 constituent studies

Constituents	Weight (%)		
	Clark et al., 1989	Douglas et al., 1993¶	Ungvary et al., 1983 and Lehotzky et al., 1985‡
Non-aromatics	0.46	< 0.10	0.04
o-xylene	2.27	3.17	2.69
m-xylene	NR	0.05	NR
p-xylene	NR	0.02	NR
Isopropylbenzene	NR	2.76	2.11
n-propylbenzene	4.05	3.95	6.99
4-ethyltoluene	16.60	6.13	31.25
3-ethyltoluene	7.14	15.85	
2-ethyltoluene	7.22	5.78	7.21
1,2,4-trimethylbenzene	32.70	39.18	33.7
1,2,3-trimethylbenzene	2.76	5.49	5.52
1,3,5-trimethylbenzene	9.35	8.09	8.80
C10	8.31*	8.32	NR
C11	NR	0.14	NR

‡ Substance reported as Aromatol (Complex C9 solvent mixture).
¶ Identical substance was used in the Schreiner et al (1989) and McKee et al (1990) studies.
NR – Proportion was not reported.
* Predominantly comprised of 1-methyl-3-n-propylbenzene, 1,2-diethylbenzene and 1-ethyl-3,5-dimethylbenzene

[2] Similarity of toxicity

Aside from the presence of unknown contaminants in the C9 aromatic fraction, the EPA had also indicated that a major reason for the exclusion of the studies on the C9 aromatic fraction was that they failed to observe clearly adverse effects (except for the reproductive/developmental toxicity study of McKee et al., 1990)[7] in contrast to the studies on the individual isomers. The implication of this statement is that there is the possibility of interactive effects where certain mixed constituents may be masking the potentially adverse effects of the trimethylbenzene isomers. However, as will be shown in this section, the data on the complex C9 aromatic fractions are virtually identical to those of the individual isomers of trimethylbenzene and ethyltoluene. For the sake of brevity, data presented on the individual constituents are limited to those present in a significant proportion in the various complex C9 fractions. For example, taking the composition of C9 fraction from Douglas et al (1993), it is immediately apparent **that isomers of trimethylbenzene, ethyltoluene, propylbenzene and xylenes make up at least**

[7] Appendix E, page E-8. Lines 27-28 of the Supplement to the Draft Assessment.

90% of the C9 fraction (Table 2). The similarity in the toxicological profile of each constituent and the complex C9 fraction would then suggest that:

- **The presence of the ≤ 10% C10-C11 component of the C9 fraction does not mask the potential toxicity of the entire C9 fraction, does not potentiate the toxic effect, and does not introduce a unique toxic effect that is not seen in the key individual constituents**.

i. Neurotoxicity

Table 3 lists the results of neurotoxicity studies on individual constituents of a typical complex C9 aromatic fraction. The results are compared with neurotoxicity and developmental toxicity tests on a complex C9 aromatic fraction. Analyses of the results, both with individual constituents or in the complex C9 aromatic fractions, show a consistent pattern of acute central nervous system (CNS) depression immediately following exposure with complete recovery following cessation of exposure.

In the Korsak studies (heavily relied upon by the EPA in the Draft Assessment), a few statistically significant responses are observed. However, many of these responses are characterized by wide variations, lack of dose-response and the complete absence of temporal concordance. As an example, Gralewicz et al. (1997) reported statistically significant effects in passive avoidance tests following a 4-week exposure to 1,2,4-trimethylbenzene in the mid and high dose groups at 48 days post exposure, 7 days after footshock (0, 123, 492, 1230 mg/m^3; 0, -20, -79*, -49%). No significant effects were observed in three other paradigms attempted (Table 1-1)[8]. However, a subsequent study (Gralewicz & Wiaderna, 2001) with 492 mg/m^3 1,2,4-trimethylbenzene reported no statistically significant effect in all four passive avoidance tests attempted. Further details regarding concerns with EPA's interpretation of the statistical significance of study results are provided in Attachment I.

In Appendix E of the Supplement to the Draft Assessment, the EPA concluded that the Douglas et al. (1993) study was not reliable because the lack of neurotoxic effects was not compatible with the neurotoxic effects of premating exposures in McKee et al. (1990) even though similar exposure concentrations were employed. In McKee et al. (1990), pregnant and non-pregnant adult mice were reported to show signs of neurotoxicity, including abnormal gait, decreased motor activity and slight ataxia, which is consistent with acute CNS depression. However, this criticism misses two key points:

a. The effects reported in McKee et al. (1990) were seen immediately after exposure. However, in the Douglas et al. (1993) study, rats were tested 48 hours **after** last-exposure to avoid confounding acute effects. An example of how time at which observations are made may

[8] Table 1-1, page 1-11 of the Draft Assessment.

affect clinical observations is noted in Table 3. In the NTP oral subchronic study of m-xylene, mice administered 2000 mg/kg-day showed acute neurological effects, including abnormal gait, tremors and ataxia (NTP, 1986); similar to those observed in inhalation-exposed mice in McKee et al. (1990). What is most important however is that in the NTP study, the observed neurological effects were only seen within the first hour of m-xylene administration, followed by complete reversal of acute CNS effects.

b. Although the highest exposure concentrations in the Douglas et al. (1993) and McKee et al. (1990) studies were identical (7500 mg/m^3), the studies employed different animal species. In Douglas et al. (1993), rats were used (standard body weight – 350 g) while McKee et al. (1990) tested mice (standard body weight – 25 g). The 14-fold difference in body weight would most likely have led to a much larger exposure/unit mass in the mouse compared to the rat.

Overall, comparison of the neurotoxicity data for both individual constituents and complex C9 aromatic substance revealed no evidence for unique differences such as to preclude the use of data on the complex C9 aromatic substances from weight of evidence considerations.

Table 3: Neurotoxicity studies on complex C9 fractions and key individual constituents

Test	Substance tested	Assay/Doses	Results	Reference
Studies with the major individual constituents of complex C9 aromatic fractions				
Acute neurotoxicity test in rats (inhalation).	1,2,3-, 1,2,4- and 1,3,5-trimethylbenzene	250 – 2000 ppm (1230 – 9840 mg/m^3) once for 4 hours.	• No deaths reported. • Dose dependent increase in response to rotarod performance test and latency to paw lick (hot plate method) **when tested immediately after exposure**.	(Korsak & Rydzynski, 1996)
Subchronic neurotoxicity test in rats (inhalation).	1,2,3- and 1,2,4-trimethylbenzene	25, 100 or 250 ppm (123, 500 or 1230 mg/m^3), 6h/day, 5 days/week for 3 months.	• No deaths reported. • No significant clinical observations made. • **Increased latency to paw lick was observed when tested immediately after last exposure.** • **No effects on latency to paw lick when tested 2 weeks after last exposure.**	(Korsak & Rydzynski, 1996)
Subacute neurotoxicity test in rats (inhalation)	1,2,3-, 1,2,4- and 1,3,5-trimethylbenzene	123 – 1230 mg/m^3 6h/day, 5 days/week for 4 weeks.	• Very few statistically significant responses when tested ≥ 2 weeks after exposure. • Wide and inconsistent variations were noted in statistically significant responses reported. These were not dose-responsive and temporal concordance could not be established for any of the responses.	(Gralewicz & Wiaderna, 2001; Gralewicz et al., 1997; Lutz et al., 2010; Wiaderna et al., 1998; Wiaderna et al., 2002)
Subacute neurotoxicity test in rats (inhalation)	m-xylene	100 ppm, 6h/day, 5 days/week for 4 weeks.	• No effect in radial maze tests 14-18 days post-exposure. • No effect on open field activity 25 days post-exposure.	(Gralewicz & Wiaderna, 2001)

			• No effect on active avoidance tests 54-60 days post-exposure. • Significant effects in 1 of 6 trials in a passive avoidance test 39-48 days post-exposure. • Significant effects in paw-lick latency only with footshock employed 50-51 days post-exposure.	
Subchronic toxicity test in mice (oral)	Technical xylene‡	125, 250, 500, 1000 or 2000 mg/kg-day, 5 days/week for 13 weeks.	• Lethargy, unsteady gait, tremors and paresis at 2000 mg/kg-day within 5-10 minutes of dosing for up to 1 hour.	(NTP, 1986)
Acute neurotoxicity test in rats (inhalation).	Isopropylbenzene	500 – 6000 mg/m^3 for 6 hours.	• Alterations in FOB with a NOAEC of 500 mg/m^3.	(Cushman et al., 1995)
Subchronic neurotoxicity test in rats (inhalation).	Isopropylbenzene	250, 500, 2500 or 6000 mg/m^3), 6h/day, 5 days/week for 3 months followed by a 4-week recovery period.	• Unsteady gait in rats within 1 hour post-exposure. No effects reported after 6 hours post-exposure. • No exposure-related changes in FOB, auditory brain stem response, brain measurements or histopathology of nervous system tissues.	(Cushman et al., 1995)
Studies with complex C9 aromatic fractions				
Subchronic neurotoxicity test in rats (inhalation).	C9 aromatic substance (See Table 2 for composition)	500, 2500 or 7500 mg/m^3), 6h/day, 5 days/week for 3 months.	• Rats tested 24-48 hrs post-exposure to avoid confounding acute effects. • No exposure-related effects on motor activity and FOB tests. • No histopathological effect on nervous system tissue.	(Douglas et al., 1993)
Acute neurotoxicity test in rats (inhalation).	Mixed isomer C9 aromatic solvent.	200, 1000 or 5000 mg/m^3 once for 8 hours each on 3 consecutive days.	• Effects on gait, hunched body positions, motor activity and slight ataxia reported within first hour after first exposure in the 5000 mg/m^3 exposure group alone. • Some statistically significant effects in the visual discrimination test were observed in the 1000 and 5000 mg/m^3 exposure groups after the first 8–hr exposure. These effects were reversed 24-hours post last exposure.	(McKee et al., 2010)
Developmental neurotoxicity test in rats (inhalation)	C9 aromatic fraction (See Table 2 for compositional information)	600, 1000 or 2000 mg/m^3, 24h/day from gestation days 7-15.	• No effects with open field spontaneous locomotive activity tests in 23, 36 and 90-day old pups. • No effects on amphetamine sensitivity tests in 37-day old pups. • No statistically significant effects in learning ability tests conducted on male pups from postnatal day 42.	(Lehotzky et al., 1985)

‡ Contains 60% m-xylene, 13.6^ p-xylene, 17% ethylbenzene and 9.1% o-xylene.

ii. Subchronic/chronic toxicity

As shown in Table 4, the systemic effects of prolonged exposures to either individual C9 alkylbenzenes or as complex C9 fractions are basically identical. Overall, the general effects include body weight decreases, mild increases in liver and kidney weights (although histopathological changes indicative of tissue injury were not observed), hematological changes (mostly decreases in RBCs with increased leukocyte counts) and upper respiratory tract irritation (associated with increased inflammatory cell counts in bronchoalveolar lavage fluid). In addition, the variations in the C8 and/or C10 alkyl benzene components in the complex fraction did not change the rodent systemic response.

Table 4: Subchronic toxicity studies on complex C9 fractions and key individual constituents

Test	Substance tested	Assay/Doses	Results	Reference
Studies with the major individual constituents of complex C9 aromatic fractions				
Subchronic toxicity in rats (inhalation)	1,2,4-trimethylbenzene	123, 492 and 1230 mg/m^3. 6 h/day, 5 days/week for 3 months	• Low-grade anemia (decreased RBC and reticulocytes at 1230 mg/m^3). • Increased SDH activity at all exposure levels. • Decreased clotting time at 492 and 1230 mg/m^3 with no dose-response pattern. • Statistically significant increase in pulmonary lesions at 492 and 1230 mg/m^3. No incidence data available.	(Korsak et al., 2000a)
Subchronic toxicity in rats (inhalation)	1,2,4-trimethylbenzene	123, 492 and 1230 mg/m^3. 6 h/day, 5 days/week for 3 months	Increase in total cell count and macrophage cell count in bronchoalveolar (BAL) fluid at all exposure levels.	(Korsak et al., 1997)
Subchronic toxicity in rats (inhalation)	1,2,3-trimethylbenzene	123, 492 and 1230 mg/m^3. 6 h/day, 5 days/week for 3 months	Increased liver weight associated with slight increase in SDH activity in high exposure male rats. Increased number of goblet cells and interstitial lung parenchyma infiltration in high exposure males and females.	(Korsak et al., 2000b)
Subchronic toxicity in rats (oral)	1,3,5-trimethylbenzene	50, 200 and 600 mg/kg/day. 5 days/week for 90 days.	Increased liver weights.	(Adenuga et al., 2014)
Subchronic toxicity in rats (oral)	*p*-ethyltoluene	100, 300 and 900 mg/kg/day. Once daily for 94 days.	• Dose-related mortality and decreased body weights. • Increased liver weights associated with increases in ALP, albumin and ALT in 300 and 900 mg/kg dose groups.	(USEPA, 2009)
Subchronic toxicity in	*p*-ethyltoluene	477 or 2337 mg/m^3, 6 h/day, 5 days/week for	• Statistically significant increase in total cells,	(Swiercz et al., 2000)

rats (inhalation)		4 weeks.	macrophages, neutrophils and lymphocytes in BAL fluid from high dose male rats. • Increased number of rats with pulmonary lesions in high exposure group.	
Subchronic toxicity in rats (inhalation)	Isopropylbenzene	492, 2438 or 5909 mg/m3 for 6 h/day, 5 days/week for 13 weeks.	• Statistically significant increase in kidney, liver and adrenal weights. • Low grade anemia with concentration-dependent increase in leukocyte count.	(Cushman et al., 1995) Virtually identical results are also reported in (Fabre et al., 1955; Jenkins et al., 1970)
Subchronic toxicity in rats (inhalation)	*m*-xylene	***Study 1*** - 100 ppm, 6 h/day, 5 days/week for 6 months or 1000 ppm for 3 months. ***Study 2*** – 50 or 100 ppm, 6 h/day, 5 days/week for 3 months	• Decreased lymphocyte differential counts and increased monocyte counts in study 1. • Low-grade anemia with increased leukocyte counts in study 2 (exposure to 100 ppm).	2 Korsak studies (Korsak et al., 1992; Korsak et al., 1994) were summarized in USEPA, 2003
Studies with complex C9 aromatic fractions				
Subchronic toxicity in rats (inhalation)	C9 aromatic substance (See Table 2 for composition)	1800, 3700 or 7400 mg/m^3 for 13 weeks.	• Increased liver and kidney weights in high exposure females. • Low-grade anemia in females at all exposure levels.	Summarized in Clark et al., 1989
Subchronic toxicity in rats (inhalation)	C9 aromatic substance (See Table 2 for composition)	450, 900 or 1800 mg/m^3, 6h/day, 5 days/week for 12 months.	• Reduced body weight gain in high exposure rats. • Increased liver and kidney weights in high exposure males. • Various statistically significant hematological changes at 6 months but not at 12 months.	(Clark et al., 1989)
Subchronic toxicity in rats (inhalation)	C9-C10 alkyl aromatic fraction‡.	200 ppm, 8h/day, 5 days/week for 90 exposures.	• No persistent or significant peripheral blood changes, weight gains, bone marrow or eye lens changes were observed in the rats.	(Nau et al., 1966)
Subchronic toxicity in rats (inhalation)	C9-C10 alkyl aromatic fraction‡.	460, 1100 or 2200 mg/m3,	• Reduced body weight in high exposure groups. • Statistically significant reduction in BUN in high exposure rats.	(Carpenter et al., 1975)

‡These fractions contained about 30-45% C9 alkylbenzenes.

iii. Developmental toxicity

As shown in table 5, there was virtually no difference in the developmental toxicity of individual constituents of C9 aromatic fractions and the complex substance. Overall, none of the constituents or complex substances caused malformations in the various species tested. Fetotoxicity appeared to be associated with maternal toxicity and as has been reported earlier, severity of maternal toxicity and fetotoxicity appeared to be influenced more by differences in study design (USEPA, 2003). For example, the highest severity of effects was seen in the studies where exposure occurred over a 24-hour time period in contrast to the more typical 6-hour exposures.

Table 5: Developmental toxicity studies on complex C9 fractions and key individual constituents

Test	Substance tested	Assay/Doses	Results	Reference
Studies with the major individual constituents of complex C9 aromatic fractions				
Developmental toxicity in rats (inhalation)	1,2,4-trimethylbenzene	100, 300, 600 or 900 ppm 6 h/day on gestational days 6-20.	• Statistically significant decrease in maternal body weight gain and food consumption from 600 ppm. • Significant reduction in fetal body weight from 600 ppm. • No evidence of teratogenic effects.	(Saillenfait et al., 2005)
Developmental toxicity in rats (inhalation)	1,3,5-trimethylbenzene	100, 300, 600 or 1200 ppm 6 h/day on gestational days 6-20.	• Statistically significant decrease in maternal body weight gain and food consumption from 300 ppm. • Significant reduction in fetal body weight from 600 ppm. • No evidence of teratogenic effects.	(Saillenfait et al., 2005)
Developmental toxicity in rats (oral)	*p*-ethyltoluene	25, 100 or 200 mg/kg/day from gestation days 6-19.	• No evidence of maternal and fetal effects.	(USEPA, 2009)
Developmental toxicity in rabbits (oral)	*p*-ethyltoluene	25, 125, 200 or 250 mg/kg/day from gestation days 6-27.	• 12/16 dams died in highest dose group. • Increased incidence of fetuses with 13th full ribs in the 125 mg/kg dose group. • Increased incidence of fetuses with 13th rudimentary rib at 200 mg/kg dose group. • No other reproductive	(USEPA, 2009)

			and fetal effects were reported. • Developmental parameters could not be evaluated meaningfully due to the high mortality in the 250 mg/kg dose group.	
Developmental toxicity in rats (inhalation)	Isopropylbenzene	487, 2399 or 5935 mg/m^3, 6h/day on gestation days 6-15.	• Statistically significant decrease in maternal body weight gain on gestation days 6-9. • No significant adverse effect on reproductive parameters and fetal development was reported.	(Darmer et al., 1997). A follow-up study in rabbits showed a non-significant increase in early resorptions and non-significant decrease in percent of live fetuses associated with a statistically significant decrease in body weight gain and increased relative liver weight in dams following exposure to 11,300 mg/m^3 isopropylbenzene (Darmer et al, 1997).
Developmental toxicity in rats (inhalation)	*o*-, *m*- and *p*-xylene	150, 1500 or 3000 mg/m^3, 24h/day from gestation day 7-14.	• The authors reported a dose-dependent increase in the incidence of fetal retardation at concentrations that caused maternal effects. • The authors reported that none of the isomers were teratogenic.	(Ungváry et al., 1980)
Developmental toxicity in rats (inhalation)	*o*-, *m*- and *p*-xylene	100, 500, 1000 or 2000 ppm (434, 2167, 4335 or 8670 mg/m^3), 6h/day for gestation days 6-20.	• No evidence of teratogenicity was found with exposure to any of the xylene isomers. • Significant decreases in fetal body weight were associated with significant decrease in maternal body weight gain and food consumption.	(Saillenfait et al., 2003)
Studies with complex C9 aromatic fractions				
Developmental toxicity in rats (inhalation)	C9 aromatic fraction (See Table 2 for compositional information)	600, 1000 or 2000 mg/m^3, 24h/day from gestation days 7-15.	• Liver weight enlargement in dams. Authors reported slight toxic effects in the dams. • Increased incidence of internal organ and skeletal retardations were reported in the	(Ungvary et al., 1983)

			fetus from 1000 mg/m^3. However, these changes had largely disappeared by post natal day 90 indicating a lack of toxicological relevance for the mild changes seen on gestation day 21.	
3-generation reproductive/Developmental toxicity in mice (inhalation)	C9 aromatic fraction (See Table 2 for compositional information)	(500, 2500, 7500mg/m^3 6hr/day from gestational days 6-15	• 1500ppm – 50% maternal mortality • 500ppm - maternal and fetal body weights reduced • 100ppm – no effects	(McKee et al., 1990)

iv. Reproductive toxicity

Unlike other endpoints, the database for reproductive toxicity of individual constituents was not as robust. A search of existing databases revealed a 2-generation reproductive toxicity study of n-butylbenzene (a C10 component of C9 aromatic fractions), a 1-generation reproductive toxicity study of mixed xylenes and a reproductive toxicity screening study of 1,4-diethylbenzene (C10 isomers present in C9 aromatic fractions at about 2%) (See Table 2). In all three cases, there were no treatment-related effects on reproductive and fertility indices. With the exception of maternal effects such as increased mortality and reductions in body weight gain, inhalation exposure to a complex C9 aromatic fraction (see Table 2 for compositional information) had no effect on reproductive and fertility indices in a 3-generation reproductive toxicity study in mice. Overall, the lack of effects in the complex C9 fraction was consistent with the lack of effects noted in the existing data on individual constituents.

Table 6: Reproductive toxicity studies on complex C9 fractions and key individual constituents

Test	Substance tested	Assay/Doses	Results	Reference
Studies with the major individual constituents of complex C9 aromatic fractions				
2-generation reproductive toxicity in rats (oral)	n-butylbenzene	30, 100 or 300 mg/kg/day over 2 generations.	• No effects on reproductive fertility in males or females.	(Izumi et al., 2005)
1-generation reproductive toxicity in rats (inhalation)	Mixed xylenes‡	60, 250 or 500 ppm, 6h/day for 131 days premating, 20 day mating period, gestation and lactation.	• No effects on pregnancy and fertility indices in males and females.	Study report summarized in (OEHHA, 2012)
Reproductive toxicity screening test in rats (oral) Similar to OECD TG (422)	1,4-diethylbenzene	30, 150 or 750 mg/kg	• No treatment-related effects on reproductive and developmental toxicity.	Robust study summary provided in (OECD, 1994)
Studies with complex C9 aromatic fractions				
3-generation reproductive/Developmental	C9 aromatic fraction (See	(500, 2500, 7500mg/m^3	• No evidence of treatment-related	(McKee et al., 1990)

toxicity in mice (inhalation)	Table 2 for compositional information)	6hr/day from gestational days 6-15	effects on reproductive and fertility indices. Maternal effects such as increased mortality and reduced body weight gain were observed in the mid and high dose groups.	

‡ Details on constituents were not provided but this was likely a mixture of xylene isomers and ethylbenzene based on compositional information on other technical xylenes.

[3] Manufacture, use and exposure considerations

As the EPA has stated, trimethylbenzene isomers are primarily "*produced during petroleum refining and production of aromatic hydrocarbons with nine carbons (i.e., C9 aromatic fraction)"* and that the "*vast majority of the C9 fraction is used as a component of gasoline*"[9]. In the presentation made by the EPA to the trimethylbenzene augmented Chemical Assessment Advisory Committee, the EPA identifies the primary use of trimethylbenzenes as part of the C9 fraction used as blending agents in gasoline formulations, as industrial solvents and as paint thinners[10]. With regard to exposure considerations, the EPA indicates that "*vehicle emissions are expected to be the major anthropogenic source of trimethylbenzenes*" and that exposures could also occur through occupational exposures in oil/gas extraction and printing industries (see footnotes).

Based on the manufacture use and exposure conditions described above, it is clear that trimethylbenzenes are primarily produced and utilized, not as individual isomers, but as part of a complex substance consisting of C9 alkylbenzenes that may also include smaller percentages of C8-C10 aromatic hydrocarbons (Firth, 2008). If this is true, then it stands to reason that the primary exposure to trimethylbenzenes would occur, not as individual isomers, but as part of a complex containing predominantly C9 isomers. This is in line with the EPA's conclusion that general population exposures to trimethylbenzenes occur through emissions from refining activities (manufacture of aromatic C9 fraction), automobile combustion (aromatic C9 fraction blended into gasoline) and in printing ink industries (where aromatic C9 fractions are used as printing ink solvents) (Firth, 2008).

The EPA indicated that data on individual trimethylbenzene isomers were used exclusively because "*current information demonstrates that trimethylbenzene isomers are released to and persist in the environment and that human populations are exposed to trimethylbenzenes in occupational and residential settings*"[11]. The EPA bolsters this argument by citing the data on the yearly emissions data on 1,2,4-trimethylbenzene. **We agree with the EPA that isomers of**

[9] Executive Summary (Occurrence and Health Effects) – Lines 2-5, page xxxiv of the Draft Assessment.
[10] Slide 4, EPA presentation on "*Overview of the Draft IRIS Assessment of Trimethylbenzenes*". May 22nd, 2014.
[11] EPA response to public comments – Appendix F, line 4-7, page F-3 of Supplement to Draft Assessment.

trimethylbenzene are indeed released into the atmosphere where potential human exposure can occur. However, it should be noted that the Toxics Release Inventory (TRI) data that the EPA cites does not take into account the source of the 1,2,4-trimethylbenzene. Based on the manufacture, use and exposure considerations outlined here, we believe that the complex C9 aromatic fraction are the primary source of the 1,2,4-trimethylbenzene. As has been outlined in prior sections, the individual trimethylbenzene isomers are structurally similar to the alkylbenzenes present in the C9 aromatic fraction and are toxicologically identical. Hence, the decision to exclude the large amount of federally mandated data on the toxicity of the complex C9 aromatics is not justified in light of these and potential exposure conditions.

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